

U.S. Ser. No. 09/673,779 -7-  
Clean Copy of Currently Pending Claims  
January 31, 2003

WHAT IS CLAIMED IS:

1. (Amended) A method for identifying the presence of a bacterium in a sample comprising
  - a) testing said sample by Gram-staining and
  - b) testing said sample with a probe according to an *in situ* hybridisation protocol selected on the basis of the outcome of said Gram-staining and identifying the presence of the bacterium in the sample.
2. A method according to claim 1 wherein said sample is a clinical sample.
3. (Amended) A method according to claim 2 wherein said sample is mammalian blood.
4. (Twice Amended) A method according to claim 1 when said Gram-staining indicates the presence of a Gram-negative bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.
5. (Amended) A method according to claim 4 wherein said character is of the rod type, further comprising hybridising said sample with at least one probe selected from a group consisting of probes capable of hybridising with nucleic acid found *Escherichia coli*, in *Klebsiella pneumoniae*, in *Klebsiella oxytoca*, in *Serratia marcescens*, in *Enterobacter aerogenes*, in *Enterobacter cloacae*, in *Proteus vulgaris*, in *Proteus mirabilis*, in *Salmonella typhi*, in *Pseudomonas aeruginosa*.

U.S. Serial No. 09/673,779  
Clean Copy of Currently Pending Claims  
January 31, 2003

-8-

7. (Twice Amended) A method according to claim 6 wherein said probe is having no more than five mismatches with a probe selected of a group consisting of probes having a sequence GCCTGCCAGTTTCGAATG (SEQ ID NO:1) or GTAGCCCTACTCGTAAGG (SEQ ID NO:2) or GAGCAAAGGTATTAACCTTACTCCC (SEQ ID NO:3) or GTTAGCCGTCCTTTCTGG (SEQ ID NO:4).

8. A method according to claim 4 wherein said character is of the coccus type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.

9. (Twice Amended) A method according to claim 1, when said Gram-staining indicates the presence of a Gram-positive bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.

10. A method according to claim 9 wherein said character is of the rod type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme and/or Proteinase K.

11. (Amended) A method according to claim 9 wherein said character is of the coccus type, further comprising determining a chain-like or clump-like character of said bacteria.

12. A method according to claim 11 wherein said character is chain-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.

13. (Amended) A method according to claim 12 further comprising hybridising said sample with at least one probe selected from a group consisting of probes capable of

*Enterococcus faecium*.

U.S. Serial No. 09/673,779  
Clean Copy of Currently Pending Claims  
January 31, 2003

-9-

14. A method according to claim 13 wherein said nucleic acid is ribosomal RNA.
15. (Twice Amended) A method according to claim 14 wherein said probe is having no more than five mismatches with a probe selected of a group composed of probes having a sequence TTATCCCCCTCTGATGGG (SEQ ID NO:5) or AGAGAAGCAAGCTTCTCGTCCG (SEQ ID NO:10) or GCCACTCCTCTTTTCCGG (SEQ ID NO:7).
16. A method according to claim 11 wherein said character is clump-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysostaphin and/or Proteinase K.
17. (Amended) A method according to claim 16 further comprising hybridising said sample with at least one probe selected from a group consisting of probes capable of hybridising with nucleic acid found in *Staphylococcus aureus*, in *Staphylococcus haemolyticus*, in *Staphylococcus saprophyticus*.
18. A method according to claim 17 wherein said nucleic acid is ribosomal RNA.
19. (Twice Amended) A method according to claim 18 wherein said probe is having no more than five mismatches with a probe selected of a group consisting of probes having a sequence GCTAATGCAGCGCGGATCC (SEQ ID NO:8) or CCGAAGGGGAAGGCTCTA (SEQ ID NO:9) or AGAGAAGCAAGCTTCTCGTCCGT (SEQ ID NO:10).

U.S. Serial No. 09/673,779  
Clean Copy of Currently Pending Claims  
January 31, 2003

-10-

20. (Twice Amended) A method according to claim 4 further comprising hybridising said sample with at least one positive control probe and/or with at least one negative control probe.

21. (Amended) A method according to claim 20 wherein said positive control probe comprising no more than five mismatches with a probe with the sequence GCTGCCTCCCGTAGGAGT (SEQ ID NO:11) and/or wherein said negative control probe comprises no more than five mismatches with a probe with the sequence ACTCCTACGGGAGGCAGC (SEQ ID NO:12).

22. (Twice Amended) A method according to claim 1 further comprising a one-step procedure of binding bacteria present in said sample to a microscopic slide and simultaneously fixing intracellular structures.

23. (Twice Amended) A method according to claim 1 wherein said probe is selected for its properties of reactivity with a selected one or more of bacterial genera and/or species including a consideration of the susceptibility to antibiotic treatment of said probe.



bacterium in said sample, further comprising determining the rod or coccus character of said bacterium.

10. A method according to claim 9 wherein said character is of the rod type, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme and/or Proteinase K.

(Amended) 11. A method according to claim 9 wherein said character is of the coccus type, further comprising determining a chain-like or clump-like character of said bacteria. (D) (C)

12. A method according to claim 11 wherein said character is chain-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysozyme.

(Amended) 13. A method according to claim 12 further comprising hybridising said sample with at least one probe selected from a group of probes capable of hybridising with nucleic acid found in *Enterococcus faecalis*, in *Streptococcus pneumoniae*, in *Streptococcus mitis*, in *Streptococcus viridans*, in *Streptococcus sanguis*, in *Enterococcus faecium*. (D) (C)

14. A method according to claim 13 wherein said nucleic acid is ribosomal RNA.

Twice (Amended) 15. A method according to claim 14 wherein said probe is having no more than five [preferably no more than two] mismatches with a probe selected of a group composed of probes having a sequence TTATCCCCCTCTGATGGG or AGACAAGCAAGCTTCTCGTCCG or GCCACTCCCTCTTTTCCCG. (SEQ ID NO: 5) (SEQ ID NO: 6) (SEQ ID NO: 7) (D) (B) (C) (E) (E)

16. A method according to claim 11 wherein said character is clump-like, further comprising subjecting said sample to treatment with a lysis buffer comprising lysostaphin and/or Proteinase K.

(Amended) 17. A method according to claim 16 further comprising hybridising said sample with at least one probe selected from a group of probes capable of hybridising with nucleic acid found in *Staphylococcus aureus*, in *Staphylococcus haemolyticus*, in *Staphylococcus saprophyticus*. (D) (D) (C)

18. A method according to claim 17 wherein said probe is

AMENDED SHEET



26. A diagnostic test kit comprising means for detecting or identifying a bacterium suspected of being present in a sample using a method according to anyone of claims 1 to 23 or using a probe according to claim 24 or 25.

Cancelled  
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AMENDED SHEET